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Vepsäläinen, Henna

2021-02-01

Vepsäläinen , H , Hautaniemi , H , Sääksjärvi , K , Leppänen , M H , Nissinen , K , Suhonen ,
E , Saha , M , Lehto , E , Ray , C , Sajaniemi , N & Erkkola , M 2021 , ' Do stressed children
have a lot on their plates? A cross-sectional study of long-term stress and diet among
Finnish preschoolers ' , Appetite , vol. 157 , 104993 . <https://doi.org/10.1016/j.appet.2020.104993>

<http://hdl.handle.net/10138/322252>

<https://doi.org/10.1016/j.appet.2020.104993>

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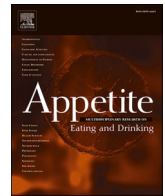
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Do stressed children have a lot on their plates? A cross-sectional study of long-term stress and diet among Finnish preschoolers

Henna Vepsäläinen^{a,*}, Hannele Hautaniemi^a, Katri Sääksjärvi^b, Marja H. Leppänen^{c,a},
Kaija Nissinen^{d,a}, Eira Suhonen^b, Mari Saha^e, Elviira Lehto^{b,f}, Carola Ray^{c,a},
Nina Sajaniemi^{b,g}, Maijaliisa Erkkola^a

^a University of Helsinki, Department of Food and Nutrition, P.O. Box 66, 00014 University of Helsinki, Helsinki, Finland

^b University of Helsinki, Department of Teacher Education, P.O. Box 9, 00014 University of Helsinki, Helsinki, Finland

^c Folkhälsan Research Center, Topeliuksenkatu 20, 00250, Helsinki, Finland

^d Seinäjoki University of Applied Sciences, Kampusranta 11, 60101, Seinäjoki, Finland

^e Tampere University, Faculty of Education and Culture, P.O. Box 700, 33014, Tampere University, Tampere, Finland

^f Tampere University, Faculty of Social Sciences, Arvo Ylpön Katu 34, 33014, Tampere University, Tampere, Finland

^g University of Eastern Finland, School of Applied Sciences and Teacher Education, P.O. Box 111, 80101, Joensuu, Finland

ARTICLE INFO

Keywords:

Fruit consumption
Healthy diet
Multi-level modelling
Physiological stress
Stress biomarker
Sugar-sweetened beverages

ABSTRACT

We examined the association between hair cortisol concentration (HCC) – an indicator of long-term stress – and diet among preschoolers in a cross-sectional design. The participants were 597 Finnish 3–6-year-olds, and the data were collected in 2015–16. We used 4-cm hair samples to analyze HCC during the past four months. Food consumption was assessed using a food frequency questionnaire, and we used consumption frequencies of selected food groups as well as data-driven dietary pattern scores in the analyses. The parents of the participating children reported their educational level and family income. The researchers measured the children's weight and height. We examined the associations between HCC and diet using multilevel linear mixed models adjusted for age, gender, the highest education in the family, household relative income, and child BMI. Higher HCCs were associated with less frequent consumption of fruit and berries (B estimate -1.17, 95% CI -2.29, -0.05) and lower scores in a health-conscious dietary pattern (B estimate -0.38, 95% CI -0.61, -0.14). Higher HCCs were also associated with more frequent consumption of sugary beverages (B estimate 1.30, 95% CI 0.06, 2.54) in a model adjusted for age, gender and highest education in the family, but the association attenuated after further adjustments. Our results are parallel with previous studies that show a link between stress and unhealthy diet. In the future, longitudinal studies are needed to establish a causal relationship between stress and diet among children.

1. Introduction

Stress has been shown to be adversely associated with health behaviors, such as physical activity, smoking, alcohol consumption and eating behavior among adults: participants with higher stress levels seem to be less physically active, smoke more, less likely to quit drinking and consume more palatable foods high in fat, salt and/or sugar (Adam & Epel, 2007; Rod et al., 2009; Steptoe et al., 1996). However, the effects

of stress on individuals' food behavior may vary according to the duration of stress (acute vs. chronic) and individual characteristics: whereas some people tend to eat less during stressful periods, others might increase their food consumption (Stone & Brownell, 1994; Torres & Nowson, 2007; Yau & Potenza, 2013). Some studies have also suggested that stress might be associated with a nutrient-dense diet (Naish et al., 2019; Torres & Nowson, 2007), and thus, stress might not only affect the amounts of food eaten, but also impair diet quality. Moreover,

Abbreviations: BMI, Body Mass Index; CI, Confidence interval; CV, Coefficients of variance; DAGIS, Increased Health and Wellbeing in Preschools; FFQ, Food frequency questionnaire; HCC, Hair cortisol concentration; PCA, Principal component analysis; SD, Standard deviation; SES, Socioeconomic status.

* Corresponding author.

E-mail addresses: henna.vepsalainen@helsinki.fi (H. Vepsäläinen), sorvari.hannele@gmail.com (H. Hautaniemi), katri.saaksjarvi@helsinki.fi (K. Sääksjärvi), marja.leppanen@folkhalsan.fi (M.H. Leppänen), kaija.nissinen@seamk.fi (K. Nissinen), eira.suhonen@helsinki.fi (E. Suhonen), mari.saha@tuni.fi (M. Saha), elviira.lehto@helsinki.fi (E. Lehto), carola.ray@folkhalsan.fi (C. Ray), nina.sajaniemi@helsinki.fi (N. Sajaniemi), majjaliisa.erkkola@helsinki.fi (M. Erkkola).

<https://doi.org/10.1016/j.appet.2020.104993>

Received 14 April 2020; Received in revised form 25 August 2020; Accepted 30 September 2020

Available online 13 October 2020

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perceived stress may also predict subsequent weight gain or obesity (Block et al., 2009; Brunner et al., 2007).

Most of the studies investigating the link between stress and diet or obesity have been conducted in adult populations. However, as prudent dietary habits, such as frequent consumption of vegetables and fruits, are typically learned during childhood and may also track into adulthood (Kelder et al., 1994; Lien et al., 2001; Mikkilä et al., 2005), childhood is a significant period for the study of food behavior and related factors, such as stress. In addition, the stress-response system is immature in young children (Loman & Gunnar, 2010), making it possible for stress to have different impacts on children's food consumption and subsequent health outcomes, such as overweight or obesity.

To date, only a handful of studies have examined the association between stress and diet among children. Studies measuring perceived stress have mainly concluded that stress correlates with the consumption of unhealthy foods, such as snacks or foods high in sugar or fat (Jenkins et al., 2005; Michels et al., 2012). The aforementioned studies have used self- and parent-reports of stress (the 20-item Feel Bad Scale (Lewis et al., 1984) and the 25-item Strengths and Difficulties Questionnaire (Goodman, 1997)). However, as self-reports are subjective by nature, researchers have started to increasingly rely on more objective indicators of stress, such as biomarkers. One such biomarker is cortisol, which can be measured from the blood, urine, saliva or hair to objectively assess stress (Vanaelst et al., 2012a). Indeed, higher salivary cortisol has been linked with more frequent consumption of sweet foods among 5–10-year-old Belgian children (Michels et al., 2013). More recently, hair cortisol concentration (HCC) has been introduced as a biomarker for chronic, long-term stress, but to the best of our knowledge, only one study has examined the relationship between HCC and food consumption: Larsen et al. reported an inverse association between HCC and fat consumption among Danish 3–7-year-olds (Larsen et al., 2019). Since dietary fat can be obtained from diverse sources, such as vegetable oils, fish and meat dishes, the study does not reveal, whether HCC is associated with healthy or unhealthy foods. In addition, no other associations between HCC and other dietary variables were found (Larsen et al., 2019).

To summarize, there seems to be a gap in the knowledge concerning the possible association between long-term stress, as assessed objectively using HCC, and diet among children. Thus, the objective of this study was to examine the association between HCC and the consumption of vegetables, fruit and berries as well as sugary foods and beverages among Finnish preschoolers. An additional aim was to determine whether HCC is related to data-driven dietary patterns describing whole-diet among the participants. We hypothesized that HCC is inversely associated with healthy and positively associated with unhealthy food consumption.

2. Methods

The current study was part of the DAGIS research project, which investigated energy balance-related behaviors, stress and related factors among Finnish preschoolers using a cross-sectional design (please see the study protocol and description of the survey process for more detail (Lehto et al., 2018; Määttä et al., 2015)). The University of Helsinki Ethical review board in humanities and social and behavioral sciences reviewed the study on February 24th 2015 and found it to be ethically acceptable (Statement 6/2015). Recruitment was performed via preschools, and 86 preschools (51% of those invited) consented to participate in the study. From these preschools, all the children in the 3–6-year-old groups ($N = 3592$) and their families were invited to participate. Preschools with a low participation rate (less than 30% of the children from all of the groups consenting; 91 children in 20 preschools) were excluded. Altogether 892 children from 66 preschools consented (25% of those invited), and data were obtained on 864 children (24% of those invited in total; 29% of those invited from the

participating preschools). Data collection took place between September 2015 and April 2016.

2.1. Hair cortisol measurements

Trained preschool personnel collected hair samples from the posterior vertex of the scalp. They used a playful approach (e.g., playing hairdresser) in the sampling. If a child felt uncomfortable during the sampling, the personnel were guided to discuss the procedure with the child and, if necessary, to terminate data collection. The hair samples consisted of approximately 40 hairs and were cut as close to the scalp as possible. If the hair could not be tied (i.e., was too short), no sample was taken. The hair samples were sent to a laboratory for analysis, packed in foil and a small plastic bag. In the laboratory, the strands were lined up and cut into two separate 2-cm segments. HCC was measured from the hair samples using chemi-luminescence immunoassay ($n = 677$, 78%) (IBL, Hamburg, Germany). The intra and inter assay coefficients of variance (CV%) were below 12% for both. In this paper, we report the mean HCC (pg/mg) of the two 2-cm segments (later referred to as 4-cm hair samples), which roughly indicates the cumulative HCC during the past four months.

2.2. Dietary assessment methods

A parent or legal guardian filled in a 47-item Food Frequency Questionnaire (FFQ) on behalf of the participating child. The parents reported how many times during the past week the child had consumed each of the foods in the FFQ. Since the parents would not have been able to assess the foods their children had eaten at preschool, the FFQ was intentionally restricted to exclude municipality-provided foods and drinks consumed during preschool hours. The FFQ had three answer columns: “not at all”, “times per week” and “times per day”. The instruction was to either tick the “not at all” box or to write a number in one of the other columns. The FFQ covered seven food groups (vegetables, fruit and berries; dairy products; fish; meat and eggs; cereal products; drinks; and other, i.e., sweets and snacks) and was based on previous studies of Finnish children (Erkkola et al., 2009; Kyttä et al., 2010) to ensure that the most important food groups were included.

2.2.1. Food consumption frequencies

Since previous studies have reported positive associations between stress and the consumption of unhealthy foods (foods high in sugar, fat and/or salt) as well as inverse associations between stress and the consumption of healthy foods (vegetables and fruit) (Jenkins et al., 2005; Michels et al., 2012, 2013; Larsen et al., 2019), we decided to focus on the consumption of sugary foods as well as vegetables and fruit, which were also specifically emphasized in the larger DAGIS study (Lehto et al., 2018; Määttä et al., 2015). Thus, we created five sum variables describing the consumption frequencies of 1) vegetables, 2) fruit and berries, 3) sugary everyday foods, 4) sugary treats and 5) sugary beverages. The sum variables were calculated for the children who had no missing information on the respective FFQ rows and are shown in Table 1 in more detail. The FFQ has shown acceptable validity in comparison to three-day food records: 69–87% of the participants were classified into the same or adjacent quarter of vegetable, fruit and berry, and sugary food consumption (Korkalo et al., 2019). The reproducibility (test-retest reliability) of the FFQ items has shown to be mostly moderate, with intraclass correlation coefficients ranging from 0.10 to 0.70 (Määttä et al., 2018).

2.2.2. Dietary patterns

We also used principal components analysis (PCA) to identify existing dietary patterns in the study sample. PCA was conducted using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA). The procedure and the resulting dietary patterns have been described earlier in more detail (Vepsäläinen et al., 2018). In brief, the 47 FFQ food items were

Table 1
Food consumption variables used as outcomes in the present study of 597 Finnish preschoolers, the DAGIS Study, 2015–2016.

| Outcome variables: consumption frequencies | FFQ rows included in the variable |
|--|---|
| Vegetables | Fresh vegetables Cooked and canned vegetables |
| Fruit and berries | Fresh fruit Berries |
| Sugary everyday foods | Flavored yogurt and quark Puddings Sugar-sweetened cereals and muesli Berry, fruit and chocolate porridge Berry and fruit stews |
| Sugary treats | Ice cream Chocolate Sweets Sweet pastries ^a |
| Sugary beverages | Sweet biscuits and cereal bars Soft drinks Flavored and sweetened milk- and plant-based drinks Sugar-sweetened juice drinks |
| Outcome variables: dietary pattern scores based on PCA | FFQ rows loading most strongly (absolute value of 0.3 or more) on each pattern |
| Sweets-and-treats | Sweet biscuits and cereal bars Chocolate Ice cream Sweets Soft drinks Sugar-sweetened juice drinks Sweet pastries ^a Crisps and popcorn Sugar-sweetened cereals and muesli Flavored nuts, almonds and seeds Sausages and frankfurters |
| Health-conscious | Plain nuts, almonds and seeds Natural yoghurt and quark Berries Eggs Wholegrain porridge and cereals Dried fruit and berries Brown rice and pasta Peas, beans, lentils and soya Cooked and canned vegetables Smoothies and fruit purées |
| Vegetables-and-processed meats | Fresh vegetables Cold cuts Fresh fruit Flavored yoghurt and quark Wholemeal bread High-fat cheese (20% or more fat) Fruit juice Sausages and frankfurters Cooked and canned vegetables Berries |

Abbreviations: FFQ, food frequency questionnaire; PCA, principal component analysis.

^a Cakes, cupcakes, sweet rolls, Danish pastries, pies and other sweet pastries.

used as input variables, and only children with no missing rows in their FFQs ($n = 758$, 88% of the participants in the DAGIS survey and 92% of the participants who provided FFQ data) were included in the PCA. The participants included in the PCA did not differ from the excluded participants in terms of age, gender, BMI, HCC or relative household income, but had parents with higher education compared to the excluded participants. Three components were chosen based on parallel inspection of eigenvalues (>1.5), scree plot and the interpretability of the components, and an orthogonal Varimax transformation was used to facilitate interpretation. We labeled these three data-driven dietary patterns on the basis of the food items that loaded most strongly into each of the patterns (Table 1). PCA describes which foods are typically used together in the sample and is not dependent on a priori assumption

concerning healthy or unhealthy diets. For each of the participants, we calculated standardized dietary pattern scores for 1) sweets-and-treats, 2) health-conscious and 3) vegetables-and-processed meats dietary patterns by assigning weights (component loadings) to the frequency of consumption of each food. The scores obtained describe how closely the food consumption of each participant mirrors each of the empirically derived dietary patterns (a higher score implying stronger adherence to a dietary pattern). Three very similar dietary patterns were also established in the same sample using three-day food records, and more than 70% of the participants were classified into the same or adjacent quarter of dietary pattern scores, demonstrating acceptable validity (Korkalo et al., 2019).

2.3. Background data & body mass index (BMI)

Gender and age at the beginning of the study were used as confounders in the present analyses. In addition, the parents recorded their educational level using six response options (comprehensive school; vocational school; secondary school; bachelor's degree or equivalent; master's degree; licentiate/doctoral degree). These categories were then recoded into three levels: secondary school or lower; bachelor's degree or equivalent; and master's degree or higher, and the highest education in the family was used as a proxy for the family's socioeconomic status (SES). The parents also reported their gross household income, which was then weighted with the number of household members and used as a confounder in the analyses.

Trained researchers measured the children's weight and height at the preschool. The participants were measured without shoes or heavy clothing using CAS portable bench scales (CAS PB-100/200). Height was measured using stadiometers (SECA 217). BMI (kg/m^2) was calculated as body weight (kg)/height² (m).

2.4. Statistical methods

We examined the associations between HCC and diet using multi-level linear mixed models (PROC MIXED of SAS Statistical package version 9.4; SAS Institute Inc., Cary, NC, USA). Since our recruitment strategy was preschool-based and could have led to clustering of the participants, we treated preschools as the highest level in the models. Because the sample included children living in the same household (altogether 95 families had two or more children participating in the study), we used the family level as the middle level. The lowest level of the three-level model was the individual participants. Preschools and families nested within these levels were considered to have fixed effects. Kenward and Roger approximation was used to calculate the denominator degrees of freedom for statistical test pertaining to fixed effects (Sterne et al., 2009).

Consumption frequencies of vegetables, fruit and berries, sugary everyday foods, sugary treats and sugary beverages, as well as standardized dietary pattern scores for sweets-and-treats, health-conscious and vegetables-and-processed meats patterns were used separately as outcomes in the models. Due to the skewness of the distribution, the possible nonlinearity of the association and the explorative nature of this study, the HCCs were categorized into fifths based on equal number of participants in each category, and the fifths were used as predictors in the models. The first fifth (the lowest HCCs) was set as the reference group. We present unstandardized B estimates and standardized β estimates for 1) the crude models (no adjustments), 2) models adjusted for age, gender and highest education in the family, and 3) models adjusted for age, gender, highest education in the family, household relative income and child BMI. The analytical sample consisted of the participants with HCC values for the 4-cm hair segments. All the children with data on at least one of the outcome variables were included in unadjusted models, and the children with data on the respective confounders were included in the adjusted models. To confirm the effect of the statistical model chosen, additional analyses using \log_{10} -transformed continuous

HCC values were also performed (data not shown). Furthermore, since multilevel Poisson regression is not implemented accessibly in statistical software, we conducted sensitivity analyses using \log_{10} -transformed food consumption frequencies as outcomes to assess robustness of the results. As the results remained unchanged (data not shown), the original food consumption frequency outcomes are presented to facilitate interpretation. HCC*gender interactions were tested, but as no statistically significant interactions were found, the results are presented for the whole sample. We used Student's *t*- and Chi-Squared tests to compare the basic characteristics and food consumption of the included and excluded children.

3. Results

The current analyses included 597 children (69%) with HCC values for 4-cm hair. The children had a mean age of 4.75 years (SD 0.91), and HCCs varied from 0.24 pg/mg to 879.60 pg/mg (median 11.69 pg/mg). Girls had lower HCCs than boys (9.75 pg/mg vs. 17.64 pg/mg, Mann-Whitney *U*-test $P < 0.001$), and the majority (62%) of the current sample were girls. Table 2 shows the descriptive statistics of the sample. Compared to the excluded children, the current sample had more children from middle-educated and less children from low-educated families (Chi-Squared test $P = 0.02$). The children included in the analyses did not differ from the excluded children in terms of age, BMI, household relative income or food consumption. HCC was not associated with BMI, household relative income or parental education.

In the unadjusted models, the children with the highest HCCs consumed vegetables (B estimate -1.62, 95% CI -3.14, -0.09), and fruit and berries (B estimate -1.50, 95% CI -2.95, -0.04) less often than the

children with the lowest HCCs (Table 3). They also scored lower on the health-conscious dietary pattern (B estimate -0.31, 95% CI -0.51, -0.11). After adjustments for age, gender and the highest education in the family, the children with the highest HCCs still seemed to consume fruit and berries less often (B estimate -1.62, 95% CI -3.09, -0.16) and scored lower on the health-conscious dietary pattern (B estimate -0.33, 95% CI -0.53, -0.14) than the children with the lowest HCCs (Table 4). In addition, the children with the highest HCCs tended to drink sugary beverages more often (B estimate 1.30, 95% CI 0.06, 2.54) than the children with the lowest HCCs. After further adjustments for household relative income and child BMI, the association between HCC and the consumption frequency of fruit and berries remained significant (B estimate -1.17, 95% CI -2.29, -0.05) (Table 5). Furthermore, the children with the highest HCCs scored lower on the health-conscious dietary pattern (B estimate -0.38, 95% CI -0.61, -0.14). The children in the middle range of HCCs (2nd–4th fifths) did not differ from the children with the lowest HCC levels in terms of food consumption. Additional analyses using continuous log-transformed HCC values as predictor yielded fairly similar results: HCC was inversely associated with the health-conscious dietary pattern (B estimate -0.11, 95% CI -0.21, -0.01 in unadjusted model; B estimate -0.12, 95% CI -0.22, -0.02 in the model adjusted with age, gender and the highest education in the family; B estimate -0.12, 95% CI -0.24, 0.00 in the model with further adjustments for household relative income and child BMI) (data not shown). A borderline significant inverse association was found between log-transformed HCC and the consumption frequency of vegetables in the unadjusted model (B estimate -0.11, 95% CI -0.22, 0.00).

Table 2

Descriptives of the 597 participants included in the present analyses, the DAGIS Study, 2015–2016.

| | Total (n = 481–597) | Girls (n = 300–371) | Boys (n = 181–226) |
|---|---------------------|---------------------|---------------------|
| Age, years, mean (SD) | 4.75 (0.91) | 4.71 (0.91) | 4.82 (0.91) |
| Missing, n (%) | 0 (0) | 0 (0) | 0 (0) |
| HCC, pg/mg, median (range) | 11.69 (0.24–879.60) | 9.75 (0.24–879.60) | 17.64 (0.46–442.64) |
| Missing, n (%) | 0 (0) | 0 (0) | 0 (0) |
| BMI, kg/m ² , mean (SD) | 15.87 (1.46) | 15.84 (1.51) | 15.92 (1.36) |
| Missing, n (%) | 32 (5) | 14 (4) | 18 (8) |
| Highest education in family, n (%) | | | |
| Master's degree or higher | 211 (35) | 122 (33) | 89 (39) |
| Bachelor's degree | 259 (43) | 167 (45) | 92 (41) |
| Secondary school or lower | 123 (21) | 78 (21) | 45 (20) |
| Missing, n (%) | 4 (1) (0) | 4 (1) (0) | 0 (0) |
| Relative household income ^a , €/month, mean (SD) | 2207 (862.7) | 2160 (873.4) | 2283 (841.5) |
| Missing, n (%) | 116 (19) | 71 (19) | 45 (20) |
| Food consumption, times/week, mean (SD) | | | |
| Vegetables ^b | 11.50 (6.75) | 11.52 (6.88) | 11.47 (6.55) |
| Missing, n (%) | 21 (4) | 10 (3) | 11 (5) |
| Fruit and berries ^c | 9.07 (6.26) | 9.12 (6.44) | 8.98 (5.97) |
| Missing, n (%) | 19 (3) | 11 (3) | 8 (4) |
| Sugary everyday foods ^d | 6.43 (5.83) | 6.38 (5.93) | 6.50 (5.66) |
| Missing, n (%) | 25 (4) | 12 (3) | 13 (6) |
| Sugary treats ^e | 6.37 (3.87) | 6.56 (4.03) | 6.06 (3.58) |
| Missing, n (%) | 23 (4) | 11 (3) | 12 (5) |
| Sugary beverages ^f | 4.10 (5.01) | 4.04 (5.13) | 4.20 (4.83) |
| Missing, n (%) | 25 (4) | 12 (3) | 13 (6) |
| Dietary pattern score, standardized score, mean (SD) | | | |
| Sweets-and-treats | 0.02 (1.01) | 0.05 (1.07) | -0.02 (0.88) |
| Health-conscious | 0.03 (1.01) | 0.06 (1.05) | -0.04 (0.93) |
| Vegetables-and-processed meats | -0.00 (0.97) | -0.02 (0.95) | 0.03 (1.00) |
| Missing, n (%) | 54 (9) | 26 (7) | 28 (12) |

Abbreviations: HCC, hair cortisol concentration.

^a Gross household income weighted with number of household members.

^b Fresh vegetables; cooked and canned vegetables.

^c Fresh fruit; fresh and frozen berries.

^d Flavored yoghurt and quark; puddings; sugar-sweetened cereals and muesli; berry, fruit and chocolate porridge with added sugar; berry and fruit stews with added sugar.

^e Ice cream; chocolate; sweets; sweet pastries; sweet biscuits and cereal bars.

^f Soft drinks; flavored and sweetened milk- and plant-based drinks; sugar-sweetened juice drink.

Table 3
Unadjusted multilevel linear mixed models with fifths of hair cortisol concentration (HCC) as predictors of food consumption frequencies (n = 572–578) and dietary pattern scores (n = 543), the DAGIS study, 2015–2016.

| | HCC, 1st fifth (0.24–3.68 pg/mg) | HCC, 2nd fifth (3.72–8.45 pg/mg) | HCC, 3rd fifth (8.51–18.26 pg/mg) | HCC, 4th fifth (18.30–56.52 pg/mg) | HCC, 5th fifth (56.63–879.60 pg/mg) |
|-------------------------------------|-------------------------------------|--|--|--|---|
| Food consumption frequencies | | | | | |
| Vegetables ^a | ref. | B estimate (95% CI) -0.73 (-2.00, 0.53) | B estimate (95% CI) -0.16 (-1.57, 1.25) | B estimate (95% CI) -1.26 (-2.71, 0.19) | B estimate (95% CI) -1.62 (-3.14, -0.09) |
| Fruit and berries ^b | ref. | β (95% CI) -0.11 (-0.30, 0.08) | β (95% CI) 0.38 (-0.98, 1.73) | β (95% CI) 0.06 (-0.16, 0.28) | β (95% CI) -0.19 (-0.40, 0.03) |
| Sugary everyday foods ^c | ref. | B estimate (95% CI) -0.30 (-1.52, 0.92) | B estimate (95% CI) 0.41 (-0.86, 1.69) | B estimate (95% CI) 0.53 (-0.86, 1.93) | B estimate (95% CI) -1.49 (-2.95, -0.04) |
| Sugary treats ^d | ref. | β (95% CI) -0.07 (-1.23, 1.08) | β (95% CI) 0.50 (-0.41, 1.41) | β (95% CI) 0.07 (-0.15, 0.29) | β (95% CI) 0.07 (-0.15, 0.29) |
| Sugary beverages ^e | ref. | B estimate (95% CI) 0.03 (-0.83, 0.88) | B estimate (95% CI) 0.79 (-0.34, 1.92) | B estimate (95% CI) 0.45 (-0.48, 1.38) | B estimate (95% CI) -0.01 (-0.96, 0.94) |
| Dietary pattern scores | | | | | |
| Sweets-and-treats | ref. | β (95% CI) 0.05 (-0.16, 0.26) | β (95% CI) 0.16 (-0.07, 0.38) | β (95% CI) 0.34 (-0.82, 1.51) | β (95% CI) 1.12 (-0.09, 2.33) |
| Health-conscious | ref. | β (95% CI) -0.01 (-0.18, 0.17) | β (95% CI) 0.08 (-0.12, 0.28) | β (95% CI) 0.09 (-0.12, 0.29) | β (95% CI) 0.02 (-0.21, 0.24) |
| Vegetables-and-processed meats | ref. | B estimate (95% CI) -0.07 (-1.23, 1.08) | B estimate (95% CI) -0.11 (-0.28, 0.07) | B estimate (95% CI) -0.11 (-0.28, 0.06) | B estimate (95% CI) -0.31 (-0.51, -0.11) |
| | ref. | β (95% CI) 0.04 (-0.14, 0.23) | β (95% CI) 0.14 (-0.07, 0.34) | β (95% CI) 0.06 (-0.15, 0.27) | β (95% CI) 0.01 (-0.22, 0.23) |

Abbreviations: HCC, hair cortisol concentration.

^a Fresh vegetables; cooked and canned vegetables.

^b Fresh fruit; fresh and frozen berries.

^c Flavored yoghurt and quark; puddings; sugar-sweetened cereals and muesli; berry, fruit and chocolate porridge with added sugar; berry and fruit stews with added sugar.

^d Ice cream; chocolate; sweets; sweet pastries; sweet biscuits and cereal bars.

^e Soft drinks; flavored and sweetened milk- and plant-based drinks; sugar-sweetened juice drink.

Table 4
Multilevel linear mixed models with fifths of hair cortisol concentration (HCC) as predictors of food consumption frequencies (n = 568–574) and dietary pattern scores (n = 539), adjusted with age, gender and highest education in family, the DAGIS study, 2015–2016.

| | HCC, 1st fifth (0.24–3.68 pg/mg) | HCC, 2nd fifth (3.72–8.45 pg/mg) | HCC, 3rd fifth (8.51–18.26 pg/mg) | HCC, 4th fifth (18.30–56.52 pg/mg) | HCC, 5th fifth (56.63–879.60 pg/mg) |
|-------------------------------------|-------------------------------------|--|--|--|--|
| Food consumption frequencies | | | | | |
| Vegetables ^a | ref. | B estimate (95% CI) -0.70 (-2.00, 0.60) | B estimate (95% CI) -0.07 (-1.53, 1.39) | B estimate (95% CI) -1.20 (-2.69, 0.28) | B estimate (95% CI) -1.48 (-3.06, 0.10) |
| Fruit and berries ^b | ref. | -0.56 (-1.78, 0.66) | 0.33 (-1.03, 1.68) | 0.50 (-0.88, 1.89) | -1.62 (-3.09, -0.16) |
| Sugary everyday foods ^c | ref. | -0.19 (-1.36, 0.98) | 0.45 (-0.85, 1.75) | 0.54 (-0.78, 1.86) | -0.07 (-1.47, 1.33) |
| Sugary treats ^d | ref. | 0.05 (-0.82, 0.91) | 0.01 (-0.21, 0.24) | 0.70 (-0.23, 1.63) | 0.18 (-0.79, 1.16) |
| Sugary beverages ^e | ref. | 0.38 (-0.69, 1.45) | 0.08 (-0.14, 0.29) | 0.81 (-0.35, 1.98) | 1.30 (0.06, 2.54) |
| Dietary pattern scores | | | | | |
| Sweets-and-treats | ref. | β (95% CI) -0.01 (-0.19, 0.17) | β (95% CI) 0.09 (-0.12, 0.30) | β (95% CI) -0.01 (-0.23, 0.21) | β (95% CI) 0.03 (-0.20, 0.26) |
| Health-conscious | ref. | -0.10 (-0.25, 0.05) | -0.12 (-0.29, 0.05) | -0.12 (-0.29, 0.05) | -0.33 (-0.53, -0.14) |
| Vegetables-and-processed meats | ref. | 0.00 (-0.19, 0.19) | 0.12 (-0.09, 0.33) | 0.12 (-0.09, 0.33) | -0.02 (-0.25, 0.21) |

Abbreviations: HCC, hair cortisol concentration.

^a Fresh vegetables; cooked and canned vegetables.

^b Fresh fruit; fresh and frozen berries.

^c Flavored yoghurt and quark; puddings; sugar-sweetened cereals and muesli; berry, fruit and chocolate porridge with added sugar; berry and fruit stews with added sugar.

^d Ice cream; chocolate; sweets; sweet pastries; sweet biscuits and cereal bars.

^e Soft drinks; flavored and sweetened milk- and plant-based drinks; sugar-sweetened juice drink.

Table 5
Multilevel linear mixed models with fifths of hair cortisol concentration (HCC) as predictors of food consumption frequencies (n = 425–438) and dietary pattern scores (n = 415), adjusted with age, gender, highest education in family, household relative income and child BMI, the DAGIS study, 2015–2016.

| | HCC, 1st fifth (0.24–3.68 pg/mg) | HCC, 2nd fifth (3.72–8.45 pg/mg) | HCC, 3rd fifth (8.51–18.26 pg/mg) | HCC, 4th fifth (18.30–56.52 pg/mg) | HCC, 5th fifth (56.63–879.60 pg/mg) |
|-------------------------------------|-------------------------------------|--|--|--|--|
| Food consumption frequencies | | | | | |
| Vegetables ^a | ref. | B estimate (95% CI) -0.07 (-0.94, 0.79) | B estimate (95% CI) -0.16 (-1.16, 0.84) | B estimate (95% CI) -0.63 (-1.73, 0.47) | B estimate (95% CI) -0.68 (-2.06, 0.69) |
| Fruit and berries ^b | ref. | -0.28 (-0.97, 0.40) | 0.22 (-0.57, 1.01) | 0.18 (-0.70, 1.06) | -1.17 (-2.29, -0.05) |
| Sugary everyday foods ^c | ref. | 0.23 (-0.86, 1.33) | 1.15 (-0.05, 2.35) | 1.03 (-0.22, 2.27) | 1.01 (-0.39, 2.41) |
| Sugary treats ^d | ref. | 0.11 (-0.74, 0.95) | 0.03 (-0.19, 0.25) | 0.73 (-0.19, 1.65) | 0.29 (-0.78, 1.37) |
| Sugary beverages ^e | ref. | 0.34 (-0.90, 1.59) | 0.07 (-0.18, 0.32) | 0.64 (-0.69, 1.98) | 1.13 (-0.35, 2.62) |
| Dietary pattern scores | | | | | |
| Sweets-and-treats | ref. | β (95% CI) 0.03 (-0.17, 0.23) | β (95% CI) 0.14 (-0.08, 0.36) | β (95% CI) 0.18 (-0.05, 0.41) | β (95% CI) 0.09 (-0.18, 0.36) |
| Health-conscious | ref. | -0.12 (-0.29, 0.05) | -0.16 (-0.35, 0.03) | -0.16 (-0.35, 0.03) | -0.38 (-0.61, -0.14) |
| Vegetables-and-processed meats | ref. | 0.03 (-0.19, 0.25) | 0.20 (-0.04, 0.43) | 0.20 (-0.04, 0.43) | 0.05 (-0.22, 0.33) |

Abbreviations: HCC, hair cortisol concentration.

^a Fresh vegetables; cooked and canned vegetables.

^b Fresh fruit; fresh and frozen berries.

^c Flavored yoghurt and quark; puddings; sugar-sweetened cereals and muesli; berry, fruit and chocolate porridge with added sugar; berry and fruit stews with added sugar.

^d Ice cream; chocolate; sweets; sweet pastries; sweet biscuits and cereal bars.

^e Soft drinks; flavored and sweetened milk- and plant-based drinks; sugar-sweetened juice drink.

4. Discussion

In this cross-sectional sample of Finnish preschoolers, higher HCCs were associated with a less healthy diet (infrequent consumption of fruit and berries as well as lower scores on a health-conscious dietary pattern). In addition, higher HCCs were associated with more frequent consumption of sugary beverages. However, this association was not statistically significant after adjusting for household relative income and child BMI. The present paper seems to be one of the first studies to examine the relationship between long-term stress and diet among preschoolers using an objective biomarker (HCC) as a stress biomarker in a relatively large sample of 3–6-year-old children.

To the best of our knowledge, the current study is the second to examine the relationship between stress and diet among children using HCC as an indicator for long-term stress. One Danish study found an inverse association between HCC and fat consumption among 3–7-year-olds (Larsen et al., 2019). However, HCC was not linked to diet quality index or the consumption of added sugar, fruit and vegetables, or sugar-sweetened beverages (Larsen et al., 2019). Thus, our results did not support this previous study. However, several methodological differences might explain the discrepancies. First, we used 4-cm hair samples, whereas Larsen et al. used 1–2-cm samples. Since hair grows approximately 1 cm per month (Vanaelst et al., 2012a), HCC was measured over a longer period in our study, which might at least partly explain the different findings. Second, the dietary assessment methods in the two studies also differed: in our study, diet was measured as consumption frequencies using FFQs, whereas Larsen et al. used four-day food records and mostly calculated nutrient intakes as outcomes. Thus, the two studies approached food consumption from different angles: we focused on food group level, which describes the behavioral aspect of food consumption (frequency of use), whereas Larsen et al. were more interested in nutritional value of the foods consumed by the participants regardless of the dietary source of the nutrients. Third, the sample in the study by Larsen et al. consisted of children predisposed to obesity (high birth weight or an overweight mother) making the samples in the two studies incomparable.

Overall, our study showed an inverse association between long-term stress and healthy diet. A similar finding among 5–12-year-old Belgian children has been reported by Michels et al., albeit they used a parent-reported questionnaire to assess stress (Michels et al., 2012). However, according to a recent review, the association between stress and healthy diet among younger children is still unclear (Hill et al., 2018), which might partly be explained by different stress indicators (perceived stress assessed using questionnaires vs. stress biomarkers such as cortisol assessing stress over shorter or longer periods of time). Since children innately prefer sweet tastes and avoid bitter-tasting foods such as vegetables (Forestell, 2017), adhering to a healthy diet requires effort from both the child and the parents. Furthermore, palatable foods (foods high in fat, salt and/or sugar) activate the reward centers in the brain (Volkow et al., 2013) making maintaining healthy diet even more challenging for families. A stressed child might not be responsive to guidance towards a healthier diet. Even though the evidence for the association between maternal stress and children's healthy diet is mixed (O'Connor et al., 2017), stressed children may live in stressed families (Bates et al., 2017), and stressed parents might not have the resources to buy, prepare and encourage their children to eat healthy foods. Future studies should examine the associations between family stress, children's stress and food consumption.

Stress and food behavior are complex phenomena and scarcely studied in children. Thus, it is possible that we were not able to cover all factors possibly related to stress or food consumption. Previous studies have shown that food-related parenting practices, such as restriction or pressure to eat, might be associated with children's eating style, food consumption and weight status (Camfferman, Van der Veek, & Rippe, 2019; Galloway, Fiorito, & Lee, 2005). To make the issue even more complicated, children's personality or temperament may also be linked

to stress sensitivity as well as to parenting practices and food consumption (Dettling, Parker, & Lane, 2000; Kaukonen, Lehto, & Ray, 2019). Thus, it is possible that multiple parent- and child-related factors may influence either children's stress or the association between stress and dietary patterns through mediation, moderation or confounding. Future studies examining these complex relationships in a longitudinal design would greatly advance our knowledge of the topic.

In our study, higher HCCs were associated with more frequent consumption of sugary beverages in a model adjusted for age, gender and highest education in the family. However, this association attenuated after adjusting for BMI, which might have been caused by the drop in sample size (the final model included 133 participants less). It is also possible that the relationship between long-term stress, BMI and the consumption of sugary beverages is more complex and cannot be fully understood in a cross-sectional design. Previous studies have also reported positive associations between stress and the consumption of unhealthy foods (foods high in salt, sugar and/or fat) (Jenkins et al., 2005; Michels, Sioen, & Braet, 2012, 2013; Hill et al., 2018), but on the other hand, obesity can also increase chronic stress (van der Valk et al., 2018). It has indeed been speculated that stress might promote irregular eating patterns and shift preferences towards foods high in fat and sugar through alterations in neuronal and/or hormonal pathways (Adam & Epel, 2007; Yau & Potenza, 2013), which could eventually result in weight gain and obesity. Indeed, higher salivary cortisol and HCC levels have been linked to higher BMI among children (Chu et al., 2017; Francis et al., 2013; Gerber et al., 2017; Rippe et al., 2016; Veldhorst et al., 2014), although not all studies have confirmed these findings (Kjölhede et al., 2014; Larsen et al., 2016; Olstad et al., 2016). As, to the best of our knowledge, even though badly needed, no longitudinal studies on the subject are available, we can only speculate on whether stress affects beverage consumption, which, in turn, affects BMI; whether beverage consumption increases BMI, which leads to increased HCCs; or whether the subject is even more complicated. We found no associations between long-term stress and the consumption of sugary everyday foods or treats, and the children with higher HCCs did not differ from the children with the lowest HCCs in terms of sweets-and-treats dietary pattern scores. A possible explanation for these findings may be the fact that young children have less autonomy over food choices and are strongly dependent on their parents as food providers and gatekeepers, and thus, their diets might not be affected by stress as much as adults' diets possibly are.

4.1. Strengths and limitations

The current study has several strengths. First of all, we used HCC as an indicator of stress in a sample of Finnish preschoolers. HCC is an objective, yet non-invasive stress biomarker, which is considered suitable for assessing long-term stress (Gow et al., 2010; Vanaelst et al., 2012b). However, it also has shortcomings: for example, the technique for analyzing HCCs has not been standardized and there are no reference values for children (Vanaelst et al., 2012a). In addition, a plethora of factors can influence HCC: the rate of hair growth, cortisol metabolism in the hair follicle and puberty, to name only a few (Vanaelst et al., 2012a; Stalder et al., 2017). To control for at least some of the confounders, studies have suggested including factors such as gender, BMI or waist circumference, last time of hair washing, parental education or family income, number of children to be supported by income, hair color, ethnicity, and the interaction between gender and hair washing frequency in the analyses (Gray, Dhana, & Van Der Vyver, 2018; Rippe, Noppe, & Windhorst, 2016). Since our sample was ethnically homogeneous and we had no information on hair washing and color, we were not able to include these as confounders in our analyses. However, the effect of hair wash frequency on HCC is controversial (Gray et al., 2018; Stalder, Steudte-Schmiedgen, & Alexander, 2017).

In the current analyses, we used a relatively large sample of 597 participants. However, as long-term stress was assessed by using the

mean HCC analyzed from 4-cm segments of hair samples, children with hair shorter than 4 cm (mostly boys) were excluded from the current analyses. Since boys had higher HCCs than girls on average, it is possible that the associations between HCC and diet in this sample were attenuated due to the lower number of boys in the sample. However, we were still able to find statistically significant associations in accordance with the hypothesis. Due to limited resources, preschools with a low participation rate (less than 30%; the cut-off had been previously agreed upon) were excluded from the study, which must be acknowledged as a possible source of bias in the study. However, since most preschools in Finland are public and children may not be admitted to the preschool closest to their home, preschools are not significantly stratified by socioeconomic status of the area. Furthermore, it is possible that parents from the most stressed and deprived families did not allow their children to participate in the study. Indeed, in the present study, at least one of the parents in 80% of the families had a bachelor's degree or higher education, whereas the corresponding percentage was 69% in the DAGIS survey sample (Lehto et al., 2018). Overall, our sample was somewhat selected, and thus the results may not be generalizable to those with lower education. However, in terms of income, another indicator of SES, the children included in the present analyses did not differ from the excluded children. We also used comprehensive adjustments in the analyses to take possible confounders, such as SES, gender and age, into account. Moreover, we used multilevel analyses to acknowledge the preschool-based sampling strategy and the fact that our sample included siblings.

4.2. Conclusions

We found a link between higher HCC, an indicator for long-term stress, and a less healthy diet among Finnish 3–6-year-olds. The current study is in line with previous studies showing mostly inverse associations between stress and healthy food consumption and positive associations between stress and unhealthy food consumption. However, the topic is still scarcely investigated, and studies published so far are heterogeneous. Thus, the results should be interpreted as preliminary and hypothesis-generating. More research using robust methods is required to elucidate the possible relationship between stress and diet among children. Furthermore, studies examining the validity and reliability of HCC as a stress biomarker are urgently needed.

Author contributions

HV, HH, KN, ES, MS, EL, CR, NS and ME conceived the study and/or contributed to the planning and the design of the study. HV, KN, MS, EL and CR collected the data and/or conducted data derivation and analyses. HV, HH, KS, MHL, KN, ES, EL, NS and ME contributed to the statistical analyses. HV wrote the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was funded by Folkhälsan Research Center, University of Helsinki, The Ministry of Education and Culture in Finland, The Ministry of Social Affairs and Health, The Academy of Finland (Grants: 285439, 287288, 288038), The Juho Vainio Foundation, The Signe and Ane Gyllenberg Foundation, The Finnish Cultural Foundation/South Ostrobothnia Regional Fund, The Päivikki and Sakari Sohlberg Foundation, Medicinska Föreningen Liv och Hälsa, Finnish Foundation for Nutrition Research, and Finnish Food Research Foundation.

Ethics approval and consent to participate

The study obtained a favorable ethical statement from the University of Helsinki Ethical review board in humanities and social and behavioral

sciences on February 24th, 2015 (Statement 6/2015). A parent or legal guardian of each participant provided an informed consent.

Acknowledgements

The authors thank the preschools, the preschool personnel, and the parents for their participation in the DAGIS study, and the research staff for data collection. In addition, the authors thank the collaborating partners of the DAGIS study for providing assistance in designing the DAGIS study.

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